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Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. GEN-T109X
Patent No. 7,041,490

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Rémy Griffais, Susan K. Hoiseth, Robert J. Zagursky, Benjamin J. Metcalf, Joel A. Peek, Banumathi Sankaran, Leah D. Fletcher
Issued	:	May 9, 2006
Patent No.	:	7,041,490
For	:	Chlamydia Trachomatis Polynucleotides and Vectors, Recombinant Host Cells, DNA Chips or Kits Containing the Same

Attention Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below are the column and line numbers where errors occurred in the patent. In the right-hand column are the page and line numbers in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 27, line 25: "ORF451;"	Page 31, line 31: -- ORF511; --

Column 43, lines 14-15: "primers; however,"	Page 51, lines 3-4: -- primers; however, --
Column 55, line 19: "F(ab')$_2$ fragments,"	Page 65, line 17: -- F(ab')$_2$ fragments, --
Column 55, line 65: "(1975, Nature 256:495497),"	Page 66, line 10: -- (1975, Nature 256:495-497), --
Column 56, lines 18-19: "PROC. NATL. ACAD. SCI."	Page 66, lines 21-22: -- PROC. NATL. ACAD. SCI. --
Column 56, lines 20-21: "1985, Nature 314:452454)"	Page 66, line 23: -- 1985, Nature 314:452-454) --
Column 69, line 34: "less than 11 ml."	Page 82, lines 20-21: -- less than 1 ml. --
Column 69, line 54: "of 101 g "	Page 82, line 32: -- of 10 μg --
Column 74, line 11: "(1% column)"	Page 88, line 13: -- (I% column) --
Column 78, line 17: "less than e^- ."	Page 93, line 24: -- less than e^{-10}. --
Column 118, lines 18-19, Table 1, ORF1020: "ID Species Score I % D64006 *Syn-* 347 44 *echocystis sp.*"	Page 145, line 10: -- ID Species Score I % D64006 *Synechocystis sp.* 347 44 --
Column 218, line 27: "Ann., Intem Med"	Page 276, line 18: -- Ann., Intern Med --
Column 221, line 23: "claim **1** or **3**, or"	Amendment Under 37 C.F.R. § 1.116, filed April 28, 2005 (claim 13, renumbered as claim 7): -- claim 1, or --

A true and correct copy of pages 31, 51, 52, 65, 66, 82, 88, 93, 145, and 276 of the application as filed and a copy of the Amendment Under 37 C.F.R. § 1.116 filed April 28, 2005, which support the Applicants' assertion of the error on the part of the Patent Office accompany this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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FCE/ehm/sl
Attachments: Certificate of Correction in duplicate
Copy of pages 31, 51, 52, 65, 66, 82, 88, 93, 145, and 276 of the application
Copy of page 3 of the Amendment Under 37 CFR 1.116 filed April 28, 2005

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on April 28, 2005.

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

AMENDMENT UNDER 37 C.F.R. § 1.116
Examining Group 1631
Patent Application
Docket No. GEN-T109X
Serial No. 09/201,228

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Ardin H. Marschel, Ph.D
Art Unit	:	1631
Applicants	:	Rémy Griffais, Susan K. Hoiseth, Robert J. Zagursky, Benjamin J. Metcalf, Joel A. Peek, Banumathi Sankaran, Leah D. Fletcher
Serial No.	:	09/201,228
Filed	:	November 30, 1998
Conf. No.	:	1303
For	:	Chlamydia Trachomatis Polynucleotides and Vectors, Recombinant Host Cells, DNA Chips or Kits Containing the Same

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 C.F.R. § 1.116

Sir:

A Petition and Fee for a one-month Extension of Time through and including April 28, 2005, accompanies this Amendment.

In response to the Office Action dated December 28, 2004, please amend the above-identified patent application as follows:

In the Claims

Claim 1 (currently amended):

An isolated polynucleotide sequence obtained from *Chlamydia trachomatis* comprising a polynucleotide sequence encoding a polypeptide selected from the group consisting of SEQ ID NO. 1083, SEQ ID NO. 1089, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, ~~SEQ ID NO. 1140,~~ SEQ ID NO. 1159, and SEQ ID NO. 1167.

Claims 2-7 (canceled)

Claim 8 (currently amended):

A polynucleotide encoding a fusion protein, comprising a polynucleotide according to Claim 1 ~~Claim 1, 2, or 3~~ ligated in frame to a polynucleotide encoding a heterologous polypeptide.

Claim 9 (currently amended):

A recombinant vector that contains the polynucleotide of Claim 1 ~~Claims 1, 2, or 3~~.

Claim 10 (original):

A recombinant vector that contains the polynucleotide of Claim 8.

Claim 11 (currently amended):

A recombinant vector that contains the polynucleotide of Claim 1 ~~Claim 1, 2, or 3~~, operatively associated with a regulatory sequence that controls gene expression.

Claim 12 (original):

A recombinant vector that contains the polynucleotide of Claim 8 operatively associated with a regulatory sequence that controls gene expression.

Claim 13 (currently amended):

A genetically engineered host cell that contains the polynucleotide of Claim 1 ~~Claim 1, 2, or 3~~, or a recombinant vector according to Claims 10 or 12.

Claim 14 (previously presented):

A genetically engineered host cell that comprises the polynucleotide of Claim 8, or a recombinant vector according to Claims 10 or 12.

Claim 15 (currently amended):

A genetically engineered host cell that contains the polynucleotide of Claim 1 ~~Claim 1, 2, or 3~~ operatively associated with a regulatory sequence that controls gene expression in the host cell.

Claim 16 (original):

A genetically engineered host cell that contains the polynucleotide of Claim 8 operatively associated with a regulatory sequence that controls gene expression in the host cell.

Claims 17-29 (canceled)

Claim 30 (currently amended):

A DNA chip containing an array of polynucleotides comprising at least one of the polynucleotides of Claim 1~~Claim 1, 2, or 3~~.

Claims 31-50 (canceled)

Claim 51 (currently amended):

A kit comprising a container containing an isolated polynucleotide of Claim 1~~, 2, or 3~~.

Claim 52 (original):

The kit of Claim 51 wherein the polynucleotide is a primer or a probe.

Claims 53-56 (canceled)

Claim 57 (previously presented):

An isolated polynucleotide comprising a polynucleotide that encodes a polypeptide sequence selected from the group consisting of SEQ ID NO. 1083, SEQ ID NO. 1089, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1159, or SEQ ID NO. 1167 wherein the polynucleotide that encodes the polypeptide sequence has the polynucleotide sequence of the genomic DNA obtainable from ECACC Deposit No. 98112618.

Claim 58 (previously presented):

An isolated polynucleotide sequence comprising a polynucleotide sequence that encodes a polypeptide sequence selected from the group consisting of SEQ ID NO. 1083, SEQ ID NO. 1089, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1159, or SEQ ID NO. 1167, wherein the polynucleotide that encodes the polypeptide sequence has the polynucleotide sequence of the clone DNA obtainable from ECACC Deposit No. 98112617.

Claim 59 (previously presented):

An isolated polynucleotide sequence comprising:

a) nucleotides 78482 to 78736 of SEQ ID NO: 1 (ORF 1083);

b) the polynucleotide sequence complementary to the full length of nucleotides 112069 through 111734 of SEQ ID NO: 1 (ORF 1089);

c) nucleotides 144164 to 144427 of SEQ ID NO: 1 (ORF 1095);

d) the polynucleotide sequence complementary to the full length of nucleotides 150698 through 150369 of SEQ ID NO: 1 (ORF 1096);

e) the polynucleotide sequence complementary to the full length of nucleotides

197313 through 197083 of SEQ ID NO: 1 (ORF 1105);

f) nucleotides 303155 to 303469 of SEQ ID NO: 1 (ORF 1117);

g) nucleotides 610110 to 610391 of SEQ ID NO: 1 (ORF 1159); or

h) the polynucleotide sequence complementary to the full length of nucleotides 679528 through 679253 of SEQ ID NO: 1 (ORF 1167).

Claim 60 (previously presented):

A recombinant vector comprising a polynucleotide according to claim 59.

Claim 61 (previously presented):

A genetically engineered host cell comprising a recombinant vector according to claim 60.

Claim 62 (previously presented):

A DNA chip comprising a polynucleotide according to claim 59.

Claim 63 (previously presented):

A polynucleotide encoding a fusion protein comprising a polynucleotide according to claim 59 ligated in frame to a polynucleotide encoding a heterologous polypeptide.

Claim 64 (previously presented):

A recombinant polynucleotide comprising a polynucleotide according to claim 59 operatively associated with a regulatory sequence that controls gene expression.

Claim 65 (previously presented):

A recombinant polynucleotide comprising a polynucleotide according to claim 63 operatively associated with a regulatory sequence that controls gene expression.

Remarks

Claims 1-3, 8-16, 30, 51, 52 and 57-65 are pending in the subject application. By this Amendment, Applicants have canceled claims 2 and 3 and amended various claims correct their dependencies in order to place this application in condition for allowance. Support for the amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1, 8-16, 30, 51, 52 and 57-65 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

As an initial matter, Applicants gratefully acknowledge the Examiner's withdrawal of the rejections under 35 U.S.C. § 112, second paragraph, and 35 U.S.C. § 102(e)(2) over Cousineau *et al.* Applicants again request the electronic records of the Patent Office be updated to reflect the new title presented in the Amendment dated March 1, 2004. A review of the Patent Office electronic records indicates the new title is not yet reflected therein.

Applicants gratefully acknowledge the Examiner's indication that claims 8, 10-12, 14-16, 51 and 52 are objected to but would be allowable if rewritten into independent form to include the limitations of any base and intervening claims. In addition, Applicants gratefully acknowledge the Examiner's indication that claims 57-65 are allowed.

Claims 1, 9, and 13 are rejected under 35 U.S.C. § 102(e)(2) as anticipated by Black. *et al.* (U.S. Patent No. 6,207,647). In addition, claims 2, 3 and 30 are rejected under 35 U.S.C. § 103(a) as obvious over Southern (WO 89/10977, 1989). Applicants have amended claims 1, 9, 13 and 30 and canceled claims 2 and 3,thereby rendering the rejection of those claims moot. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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Preferably, the invention also relates to the nucleotide sequences according to the invention, characterized in that they encode a *Chlamydia trachomatis* polypeptide or one of its representative fragments which is involved in the synthesis of the wall, such as for example KDO transferase, and the proteins responsible for the attachment of certain sugars onto the exposed proteins, and in that they comprise a nucleotide sequence chosen from the following sequences:
ORF87; ORF196; ORF242; ORF269; ORF628; ORF629; ORF634; ORF635; ORF637; ORF638; ORF1019 and one of their representative fragments.

Preferably, the invention also relates to the nucleotide sequences according to the invention, characterized in that they encode a *Chlamydia trachomatis* polypeptide or one of its representative fragments which is involved in the transcription, translation and/or maturation process, such as for example initiation factors, RNA polymerases or certain chaperone proteins, and in that they comprise a nucleotide sequence chosen from the following sequences:
ORF112; ORF113; ORF332; ORF212; ORF213; ORF350; ORF362; ORF363; ORF364; ORF407; ORF451; ORF546; ORF643; ORF744; ORF746; ORF833; ORF868; ORF981; ORF982; ORF1003; ORF1011; ORF1042 and one of their representative fragments.

Preferably, the invention also relates to the nucleotide sequences according to the invention, characterized in that they encode a *Chlamydia trachomatis* ribosomal polypeptide or one of its representative fragments, such as for example the ribosomal proteins L21, L27 and S10, and in that they comprise a nucleotide sequence chosen from the following sequences:
ORF114; ORF115; ORF116; ORF328; ORF361; ORF375; ORF445; ORF543; ORF584; ORF585; ORF743; ORF813; ORF941; ORF942; ORF944; ORF946; ORF947; ORF948; ORF950; ORF951; ORF952; ORF953; ORF954; ORF955; ORF955; ORF957; ORF958; ORF960; ORF961; ORF1040; ORF1041; ORF1043; ORF1063; ORF1064 and one of their representative fragments.

Preferably, the invention also relates to the nucleotide sequences according to the invention, characterized in that they encode a *Chlamydia trachomatis* transport polypeptide or one of its representative fragments, such as for example the proteins for transporting amino acids, sugars and certain oligopeptides, and in that they comprise a nucleotide sequence chosen from the following sequences:
ORF6; ORF50; ORF51; ORF80; ORF125; ORF126; ORF128; ORF129; ORF215; ORF246; ORF248; ORF249; ORF251; ORF252; ORF253; ORF255; ORF271; ORF275; ORF293; ORF309; ORF323; ORF324; ORF398; ORF401; ORF449; ORF511; ORF512; ORF564; ORF565; ORF667; ORF679; ORF680; ORF711; ORF712; ORF713; ORF714; ORF715; ORF730; ORF731; ORF736; ORF737; ORF738; ORF870; ORF908; ORF919; ORF977; ORF987; ORF988; ORF992; ORF993; ORF994; ORF1028; ORF1029 and one of their representative fragments.

Preferably, the invention also relates to the nucleotide sequences according to the

primer according to the invention, characterized in that they are labelled with a radioactive compound or with a nonradioactive compound.

The nonlabelled nucleotide sequences may be used directly as probes or primers; however, the sequences are generally labelled with a radioactive element (^{32}P, ^{35}S, ^{3}H, ^{125}I) or with a nonradioactive molecule (biotin, acetylaminofluorene, digoxigenin, 5-bromo-deoxyuridine, fluorescein) so as to obtain probes which can be used in numerous applications.

Examples of nonradioactive labelling of nucleotide sequences are described, for example, in French patent No. 78,10975 or by Urdea et al. or by Sanchez-Pescador et al. in 1988.

In the latter case, one of the labelling methods described in patents FR-2 422 956 and FR-2 518 755 may also be used.

The invention also relates to the nucleotide sequences of fragments which can be obtained by hybridization with the aid of at least one probe according to the invention.

The hybridization technique may be performed in various ways (Matthews et al., 1988). The most common method consists in immobilizing the nucleic acid extracted from *C. trachomatis* cells on a support (such as nitrocellulose, nylon, polystyrene) and in incubating, under well-defined conditions, the target nucleic acid immobilized with the probe. After hybridization, the excess probe is removed and the hybrid molecules formed are detected by the appropriate method (measurement of the radioactivity, of the fluorescence or of the enzymatic activity linked to the probe).

The invention also comprises the nucleotide sequences according to the invention, characterized in that they are covalently or noncovalently immobilized on a support.

According to another advantageous embodiment of the nucleic sequences according to the invention, the latter may be used immobilized on a support and may thus serve to capture, through specific hybridization, the target nucleic acid obtained from the biological sample to be tested. If necessary, the solid support is separated from the sample and the hybridization complex formed between the so-called capture probe and the target nucleic acid is then detected by means of a second probe, called detection probe, labelled with an easily detectable element.

The nucleotide sequences according to the invention may also be used in new analytical systems, DNA chips, which allow sequencing, the study of mutations and of the expression of genes, and which are currently of interest given their very small size and their high capacity in terms of number of analyses.

The principle of the operation of these chips is based on molecular probes, most often oligonucleotides, which are attached onto a miniaturized surface, generally of the order of a few square centimetres. During an analysis, a sample containing fragments of a target nucleic acid to be analysed, for example DNA or RNA labelled, for example, after amplification, is deposited onto the

DNA chip in which the support has been coated beforehand with probes. Bringing the labelled target sequences into contact with the probes leads to the formation, through hybridization, of a duplex according to the rule of pairing defined by J.D. Watson and F. Crick. After a washing step, analysis of the surface of the chip allows the effective hybridizations to be located by means of the signals emitted by the labels tagging the target. A hybridization fingerprint results from this analysis which, by appropriate computer processing, will make it possible to determine information such as the presence of specific fragments in the sample, the determination of sequences and the presence of mutations.

The chip consists of a multitude of molecular probes, precisely organized or arrayed on a solid support whose surface is miniaturized. It is at the centre of a system where other elements (imaging system, microcomputer) allow the acquisition and interpretation of a hybridization fingerprint.

The hybridization supports are provided in the form of flat or porous surfaces (pierced with wells) composed of various materials. The choice of a support is determined by its physicochemical properties, or more precisely, by the relationship between the latter and the conditions under which the support will be placed during the synthesis or the attachment of the probes or during the use of the chip. It is therefore necessary, before considering the use of a particular support (R.S. Matson et al., 1994), to consider characteristics such as its stability to pH, its physical strength, its reactivity and its chemical stability as well as its capacity to nonspecifically bind nucleic acids. Materials such as glass, silicon and polymers are commonly used. Their surface is, in a first step, called «functionalization», made reactive towards the groups which it is desired to attach thereon. After the functionalization, so-called spacer molecules are grafted onto the activated surface. Used as intermediates between the surface and the probe, these molecules of variable size render unimportant the surface properties of the supports, which often prove to be problematic for the synthesis or the attachment of the probes and for the hybridization.

Among the hybridization supports, there may be mentioned glass which is used, for example, in the method of in situ synthesis of oligonucleotides by photochemical addressing developed by the company Affymetrix (E.L. Sheldon, 1993), the glass surface being activated by silane. Genosensor Consortium (P. Mérel, 1994) also uses glass slides carrying wells 3 mm apart, this support being activated with epoxysilane.

Polymers or silicon may also be mentioned among these hybridization supports. For example, the Andrein Mirzabekov team has developed a chip consisting of polyacrylamide squares polymerized on a silanized glass surface (G. Yershov et al., 1996). Several teams use silicon, in particular the IFOS laboratory of Ecole Centrale of Lyon which uses a silicon semiconductor substrate which is p-doped by introducing it into its crystalline structure atoms whose valency is different from

- a polypeptide according to the invention,
- where appropriate, the reagents for constituting the medium appropriate for the immunological or specific reaction,
- the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction between the polypeptide(s) of the invention and the antibodies which may be present in the biological sample, it being possible for these reagents also to carry a label, or to be capable of being recognized in turn by a labelled reagent, more particularly in the case where the polypeptide according to the invention is not labelled,
- where appropriate, a reference biological sample (negative control) free of antibodies recognized by a polypeptide according to the invention,
- where appropriate, a reference biological sample (positive control) containing a predetermined quantity of antibodies recognized by a polypeptide according to the invention.

According to the invention, the polypeptides, peptides, fusion proteins or other derivatives, or analogs thereof encoded by a polynucleotide sequence in SEQ ID No. 1, may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such antibodies may include, but are not limited to, polyclonal and monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. In a specific embodiment, the antibody to a polypeptide, peptide or other derivative, or analog thereof encoded by a polynucleotide sequence in SEQ ID No. 1 is a bispecific antibody (see generally, *e.g.* Fanger and Drakeman, 1995, *Drug News and Perspectives* 8: 133-137). Such a bispecific antibody is genetically engineered to recognize both (1) an epitope and (2) one of a variety of "trigger" molecules, *e.g.* Fc receptors on myeloid cells, and CD3 and CD2 on T cells, that have been identified as being able to cause a cytotoxic T-cell to destroy a particular target. Such bispecific antibodies can be prepared either by chemical conjugation, hybridoma, or recombinant molecular biology techniques known to the skilled artisan.

Various procedures known in the art may be used for the production of polyclonal antibodies to a polypeptide, peptide or other derivative, or analog thereof encoded by a polynucleotide sequence in SEQ ID No. 1. For the production of antibody, various host animals can be immunized by injection with a polypeptide, or peptide or other derivative, or analog thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants, depending on the host species, may be used to increase the immunological response, including but not limited to Stimulon™ QS-21 (Aquila Biopharmaceuticals, Inc., Framingham, MA), MPL™ (3-O-deacylated monophosphoryl lipid A; RIBI ImmunoChem Research, Inc., Hamilton, MT), aluminum phosphate, IL-12 (Genetics Institute, Cambridge, MA), Freund's (complete and incomplete), mineral gels such as aluminum hydroxide,

surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, BCG (bacille Calmette-Guerin), and corynebacterium parvum. Alternatively, polyclonal antibodies may be prepared by purifying, on an affinity column onto which a polypeptide according to the invention has been previously attached, the antibodies contained in the serum of patients infected with a bacterium belonging to the species *Chlamydia trachomatis*.

For preparation of monoclonal antibodies directed toward a polypeptide, peptide or other derivative, or analog, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing technology described in PCT/US90/02545. In another embodiment of the invention, transgenic non-human animals can be used for the production of human antibodies utilizing technology described in WO 98/24893 and WO 96/33735. According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote *et al.*, 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of «chimeric antibodies», (Morrison *et al.*, 1984, PROC. NATL. ACAD. SCI. U.S.A. 81:6851-6855; Neuberger *et al.*, 1984, Nature 312:604-608; Takeda *et al.*, 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for a polypeptide, peptide or other derivative, or analog together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce polypeptide or peptide-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse *et al.*, 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for polypeptides, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be

KCl, 5 mM $MgCl_2$) to which 800 units of DNAse I (Boehringer) are added. The whole is kept at 37°C for one hour. One ml of 0.5 M EDTA is then added, and the whole is vortexed and frozen at -20°C.

Preparation of the DNA

The Chlamydiae purified above are thawed and subjected to a proteinase K (Boehringer) digestion in a final volume of 10 ml. The digestion conditions are the following: 0.1 mg/ml proteinase K, 0.1 % SDS at 55°C, stirring every 10 min. The product of digestion is then subjected to a double extraction with phenol-chloroform, two volumes of ethanol are added and the DNA is directly recovered with a Pasteur pipette having one end in the form of a hook. The DNA is dried on the edge of the tube and then resuspended in 500 µl of 2 mM Tris pH 7.5. The DNA is stored at 4°C for at least 24 hours before being used for the cloning.

Cloning of the DNA

After precipitation, the DNA is quantified by measuring the optical density at 260 nm. Thirty µg of Chlamydia DNA are distributed into 10 tubes of 1.5 ml and diluted in 300 µl of water. Each of the tubes is subjected to 10 applications of ultrasound lasting for 0.5 sec in a sonicator (Unisonix XL2020). The contents of the 10 tubes are then grouped and concentrated by successive extractions with butanol (Sigma B1888) in the following manner: two volumes of butanol are added to the dilute DNA mixture. After stirring, the whole is centrifuged for five minutes at 2500 rpm and the butanol is removed. This operation is repeated until the volume of the aqueous phase is less than 1 ml. The DNA is then precipitated in the presence of ethanol and of 0.5 M sodium acetate pH 5.4, and then centrifuged for thirty minutes at 15,000 rpm at cold temperature (4°C). The pellet is washed with 75% ethanol, centrifuged for five minutes at 15,000 rpm and dried at room temperature. A tenth of the preparation is analysed on a 0.8% agarose gel. Typically, the size of the DNA fragments thus prepared is between 200 and 8000 base pairs.

To allow the cloning of the DNA obtained, the ends are repaired. The DNA is distributed in an amount of 10 µg/tube, in the following reaction medium: 100 µl final volume, 1 H buffer (Biolabs 201L), 0.5 µl BSA 0.05 mg/ml, 0.1 mM dATP, 0.1 mM each of dGTP, dCTP or dTTP, 60,000 IU T4 DNA polymerase. The reaction is incubated for thirty minutes at 16°C. The contents of each of the tubes are then grouped before carrying out an extraction with phenol-chloroform and then precipitating the aqueous phase as described above. After this step, the DNA thus prepared is phosphorylated. For that, the DNA is distributed into tubes in an amount of 10 µg per tube, and then in a final volume of 50 µl, the reaction is prepared in the following manner: 1 mM ATP, 1 x kinase buffer, 10 IU T4 polynucleotide kinase (Biolabs 201L). The preparation is incubated for thirty minutes at 37°C. The contents of the tubes are combined and a phenol-chloroform extraction

Legend to Table 1: Open reading frames are identified with the GenMark software version 2.3A (GenePro), the template used is *Chlamydia trachomatis* of order 4 on a length of 196 nucleotides with a window of 12 nucleotides and a minimum signal of 0.5. These reading frames are numbered in order of appearance on the chromosome, starting with ORF2 (ORF column). The positions of the beginning and of the end are then given in column 2 (position). When the position of the beginning is greater than the position of the end, this means that the region is encoded by the strand complementary to the sequence which was given in the sequence SEQ ID No. 1.

All the putative products were subjected to a search for homology on GENPEPT (release 103 for SEQ ID No. 2 to SEQ ID No. 1076 and release 108 for SEQ ID No. 1077 to SEQ ID No. 1197 with the BLASTp software (Altschul et al. 1990), with, as parameters, the default parameters with the exception of the expected value E set at 10^{-5} (for SEQ ID No. 2 to SEQ ID No. 1076) and P value set at e^{-10} (for SEQ ID No. 1077 to SEQ ID No. 1197). Subsequently, only the identities greater than 30% (I% column) were taken into account. The description of the most homologous sequence is given in the Homology column; the identifier for the latter sequence is given in the ID column and the animal species to which this sequence belongs is given in the Species column. The Homology score is evaluated by the sum of the blast scores for each region of homology and reported in the Score column. Table 1 also reflects data from additional ORF finder programs as defined below.

Materials and methods: transmembrane domains:

The DAS software was used as recommended by the authors (Cserzo et al., 1997).

This method uses, to predict the transmembrane domains, templates derived from a sampling of selected proteins. All the regions for which a «Cutoff» greater than 1.5 was found by the program were taken into account.

Additional ORF Finder Programs

For this analysis, two additional ORF finder programs were used to predict potential open reading frames of a minimum length of 74 amino acids; Glimmer (Salzberg, S.L., Delcher, A., Kasif, S., and W. White. 1998. Microbial gene identification using interpolated Markov models. Nucleic Acids Res. 26:544-548.), and an in-house written program. The in-house program used a very simple search algorithm. The analysis required the that the genomic DNA sequence text be in the 5' to 3' direction, the genome is circular, and that TAA, TAG, and TGA are stop codons. The search parameters were as follows:

(1) A search for an ORF that started with a GTG codon was performed. If no GTG codons were found, then a search for an ATG codon was performed. However, if a GTG codon was found,

pathway in Gram-negative bacteria. Microbiol. Rev. 57:50-108) contributed toward reaching the minimum score of three.

A list of ORFs in the *Chlamydia trachomatis* genome encoding putative lipoproteins is set forth above in the specification.

LPS-Related ORFs of *Chlamydia trachomatis*

Lipopolysaccharide (LPS) is an important major surface antigen of Chlamydia cells. Monoclonal antibodies (Mab) directed against LPS of *Chlamydia pneumoniae* have been identified that can neutralize the infectivity of *Chlamydia pneumoniae* both *in vitro* and *in vivo* (Peterson et al. 1988). Similar results are expected utilizing monoclonal antibodies against LPS of *Chlamydia trachomatis*. LPS is composed of lipid A and a core oligosaccharide portion and is phenotypically of the rough type (R-LPS) (Lukacova, M., Baumann, M., Brade, L., Mamat, U., Brade, H. 1994. Lipopolysaccharide Smooth-Rough Phase Variation in Bacteria of the Genus *Chlamydia*. Infect. Immun. June 62(6):2270-2276.) The lipid A component is composed of fatty acids which serve to anchor LPS in the outer membrane. The core component contains sugars and sugar derivatives such as a trisaccharide of 3-deoxy-D-manno-octulosonic acid (KDO) (Reeves, P.R., Hobbs, M., Valvano, M.A., Skurnik, M., Whitfield, C., Coplin, D., Kido, N., Klena, J., Maskell, D., Raetz, C.R.H., Rick, P.D. 1996. *Bacterial Polysaccharide Synthesis and Gene Nomenclature* pp. 10071-10078, Elsevier Science Ltd.). The KDO gene product is a multifunctional glycosyltransferase and represents a shared epitope among the Chlamydia. For a review of LPS biosynthesis see, e.g., Schnaitman, C.A., Klena, J.D. 1993. Genetics of Lipopolysaccharide Biosynthesis in Enteric Bacteria. Microbiol. Rev. 57:655-682.

A text search of the ORF Blastp results identified several genes that are involved in Chlamydial LPS production with a P score less than e^{-10}. The following key-terms were used in the text search: KDO, CPS (Capsular Polysaccharide Biosynthesis), capsule, LPS, rfa, rfb, rfc, rfe, rha, rhl, core, epimerase, isomerase, transferase, pyrophosphorylase, phosphatase, aldolase, heptose, manno, glucose, lpxB, fibronectin, fibrinogen, fucosyltransferase , lic, lgt, pgm, tolC, rol, ChoP, phosphorylcholine, waaF, PGL-Tb1. A list of ORFs in the *Chlamydia trachomatis* genome encoding putative polypeptides involved in LPS biosynthesis is set forth above in the specification.

Type III And Other Secreted Products

Type III secretion enables gram-negative bacteria to secrete and inject pathogenicity proteins into the cytosol of eukaryotic host cells (Hueck, C. J., 1998. Type III Protein Secretion Systems in Bacterial Pathogens of Animals and Plants. In Microbiology and Molecular Biology Reviews. 62:379-433.) These secreted factors often resemble eukaryotic signal transduction factors,

ORF	begin	stop	Homology	ID	*Species*	Score	I%
ORF1014	978619	978984	spore coat protein CotRC	D50551	*Bacillus subtilis*	355	52
ORF1015	978933	979331	hypothetical	U32717	*Haemophilus influenzae*	199	40
ORF1016	981197	979389	putative				
ORF1017	979711	980112	putative				
ORF1018	982116	981148	putative				
ORF1019	982321	983598	UDP-N-acetylglucosamine enolpyruvyl transferase (murZ)	U32788	*Haemophilus influenzae*	593	38
ORF1020	984488	983862	arginyl-tRNA-synthetase	D64006	*Synechocystis sp.*	347	44
ORF1021	985381	984371	arginyl-tRNA-synthetase	D64006	*Synechocystis sp.*	782	58
ORF1022	986103	985399	hypothetical protein	D90915	*Synechocystis sp.*	224	35
ORF1023	986693	986046	No definition line found	U00021	*Mycobacterium leprae*	286	50
ORF1024	987607	986693	o298; This 298 aa ORF is 33 pct identical (24 gaps) to 248 residues of an approx. 256 aa protein CDSA_ECOLI SW: P06466	AE000238	*Escherichia coli*	132	46
ORF1025	988119	987616	conserved hypothetical protein	AE000627	*Helicobacter pylori*	343	49
ORF1026	988253	987936	hypothetical protein (HI0920)	U67577	*Methanococcus jannaschii*	110	38
ORF1027	988831	989163	putative				
ORF1028	989693	993442	protein-export membrane protein SecD	D64000	*Synechocystis sp.*	447	38
ORF1029	993408	993785	protein-export membrane protein	U83136	*Rhodobacter sphaeroides*	240	43
ORF1030	993835	993416	putative				
ORF1031	993882	994262	putative				
ORF1032	994226	995656	RecJ recombination protein	U41759	*Chlamydia psittaci*	880	66
ORF1033	996036	996611	unknown	U41759	*Chlamydia psittaci*	533	75
ORF1034	996885	998267	glutamyl-tRNA synthetase homolog	U41759	*Chlamydia psittaci*	2018	83

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,041,490
APPLICATION NO. : 09/201,228
ISSUE DATE : May 9, 2006

INVENTOR(S) : Rémy Griffais, Susan K. Hoiseth, Robert J. Zagursky, Benjamin J. Metcalf, Joel A. Peek, Banumathi Sankaran, Leah D. Fletcher

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 27,
Line 25, "ORF451;" should read -- ORF511; --.

Column 43,
Lines 14-15, "primers;
however," should read -- primers; however, --.

Column 55,
Line 19, "F(ab')$_2$ fragments," should read -- F(ab')$_2$ fragments, --.
Line 65, "(1975, Nature 256:495497)," should read -- (1975, Nature 256:495-497), --.

Column 56,
Lines 18-19, "PROC.
NATL. ACAD. SCI." should read -- PROC. NATL. ACAD. SCI. --.
Lines 20-21, "1985, Nature 314:452454)" should read -- 1985, Nature 314:452-454) --.

Column 69,
Line 34, "less than 11 ml." should read -- less than 1 ml. --.
Line 54, "of 101 g" should read -- of 10 μg --.

Column 74,
Line 11, "(1% column)" should read -- (I% column) --.

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Column 118,
Lines 18-19, Table 1, ORF1020

"ID	Species	Score	I %
D64006 *Syn-echocystis sp.*"	347	44	

should read

-- ID	Species	Score	I %
D64006	*Synechocystis sp.*	347	44 --.

Column 218,
Line 27, "Ann., Intem Med" should read -- Ann. Intern Med. --.

Column 221,
Line 23, "claim **1** or **3**, or" should read -- claim **1**, or --.

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